ON THE TOXIC EFFECT OF DELETERIOUS AGENTS ON THE GERMINATION AND DEVELOPMENT OF CERTAIN FILAMENTOUS FUNGI.

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(*Concluded from p. 327.*)

ALL acids tested retard germination and early mycelial development of the mold fungi. In the case of the mineral acids this retarding action is usually evident in $\frac{n}{1024}$ concentration. The acetic acids do not have any perceptible influence at this dilution. Cultures which are only slightly retarded almost invariably take on new vigor a few hours after germination, and overtake and surpass the checks in development of mycelium. This excessive mycelial development is usually accompanied by retardation of fruiting, and usually reaches its maximum in cultures two or three removes from the inhibiting concentration. Cultures in the acetic acids show a greater stimulation of mycelial development than in HCl or H_2SO_4. Cultures in HNO_3 resembled those in the acetic acids in development of mycelium but were not so greatly retarded in fruiting.

Smaller quantities of acid on an average proved injurious to Œdocephalum than to the other forms, $\frac{20n}{2048}$ being on the average distinctly detrimental. In this respect the others came in the following order: Botrytis, 23; Penicillium, 28; Aspergillus, 42; Sterigmatocystis, 64.

Œdocephalum was also the most easily inhibited, its relative inhibiting coefficient being 72; followed by Botrytis, 76; Penicillium, 100; Aspergillus, 104; and Sterigmatocystis, 200.

Botrytis, however, was the most easily killed. The order in this respect was: Botrytis, 100; Œdocephalum, 137; Aspergillus, 272; Sterigmatocystis, 369; and finally Penicillium, whose spores showed by far the greatest resistance, represented by the

coefficient 498. This high resistance of Penicillium in regard to the death-point may be partly due to the tendency of the spores to adhere in bunches in making the inoculation, a difficulty never fully overcome.

In table II a comparison of the chemical affinities of the different acids tested by seven methods is given.

TABLE II.

CHEMICAL AFFINITIES OF ACIDS.

HCN	Tri.	Di.	Mono.	Acetic	$\frac{1}{2}H_2SO_4$	HNO_3	HCl	Acid
—	62.3	25.3	4.9	1.4	65.1	99.6	100	Relative ionization in $\frac{n}{1}$ sol.
—	68	23	4.3	.34	74	92	100	Catalysis of methyl acetate
—	63	18	5.1	1	67	110	100	Catalysis of calcium oxalate
—	82	34	7.2	1.2	68	102	100	Neutralizing hydroxids
—	75.4	27	4.9	.4	73	100	100	Invertion of cane sugar
—	96.7	62.4	17.2	4.7	71.9	99	100	Multirotations of dextrose
200+	200	100+	100	25	100	100	100	Physiological action on Lupinus
7666	255	359	396	277	112	163	100	Ditto, on molds

Line 1 gives the relative ionization of these acids (except HCN) in normal solution. HCl being the basis of comparison here as in the succeeding tests is given 100 units. Line 2 gives the relative powers of the different acids to promote the well known catalysis of methyl acetate in aqueous solution. Line 3 gives their relative activity in decomposing calcium oxalate. Line 4 gives Ostwald's ('91) determinations of their relative affinities for hydroxids. Line 5 gives their relative activity in inverting cane sugar.

The correspondence of the results presented on lines 2–5 with the ionization data on line 1 is certainly quite striking. Arrhenius ('83) was the first to point out this close numerical agreement. Since the publication of his work in 1883 the idea has taken firm hold on many chemists that the ionized portion of an acid and that only is chemically active. Whetham ('95)

expresses concisely recent views when he says (p. 165): "We may take it, then, that only that portion of a body is chemically active which is electrolytically active — that ionization is necessary for chemical activity just as it is necessary for electrolytic conductivity."

What applies to chemical activity must also apply to physiological activity, for in its ultimate analysis the latter is doubtless due to the former. Kahlenberg and True ('96) remark (p. 35): "It has always been taken as axiomatic that the physiological action of any substance is due to its chemical character."

The first work which deviated irreconcilably from the theory that all acids have "specific coefficients of affinity . . . . based on the fact that the relative affinities of different acids are the same, whatever the nature of the action by which they are compared" (Whetham, '95, p. 162) was that of Levy ('95). It will be seen (line 6) that acetic, mono- and dichloracetic give coefficients of activity which are in round numbers 200 per cent. in excess of that called for by the theory. The physiological activity of the acids towards phanerogams (Kahlenberg and True '96) (line 7) is equally out of harmony with the theory, when we find the almost un-ionized HCN much more active than the "strong" mineral acids. The climax, however, is reached in the data recorded on line 8, where we are dealing with concentrations which contain in many cases a very large proportion of un-ionized molecules.

The chemical reactions involved in physiological investigations are doubtless vastly more complex than in the case of the earlier studies recorded on lines 2–5. In the data recorded on lines 6–8, derived from the action of the acids on the more complex carbon compounds, and the highly complex aldehydes, albuminoids, etc., found in the protoplasm of living cells, we surely find a great exception to the alleged law that the relative affinities of different acids are the same *whatever the nature of the action by which they are compared.* These affinities, indeed, appear in some cases to be almost the converse of that required by the theory above noted.

HYDROXIDS.

As it is quite impossible to handle solutions of hydroxids in an ordinary atmosphere without a greater or less loss by neutralization by the CO_2 of the air, the following toxic values of potassium hydroxid and ammonia must be somewhat less than their absolute toxic value. This source of error was reduced as much as possible by rapid handling in making the cultures.

Potassium hydroxid, KOH; 77 (?), *166*, 282. In no other case was it found so difficult to determine where to place the coefficient of injury. $\frac{n}{512}$ retarded germination in all cases, and with some forms $\frac{n}{1024}$ and even $\frac{n}{2048}$ (Œdocephalum) showed an injurious influence.

At 24 and 36 hours, however, the cultures presented a very different appearence. Cultures in $\frac{n}{2048}$ concentration showed a heavier mycelium than the checks, and with stronger concentrations this stimulation of mycelial development was more marked until the climax was reached in $\frac{n}{64}$ or $\frac{n}{32}$ concentration.

As $\frac{n}{12}$ proved the average inhibiting point, it will be seen that, as with the acids, the maximum stimulation of mycelial development occurred about two removes from the limit of germination, or, in other words, in solutions containing one fourth the concentration of the agent inhibiting the germination of the spores. The retardation of fruiting in the stimulated cultures was very marked, and suggested the query as to whether they were both due to the same cause, or whether one was a result of the other. It is well known that with the higher plants suppression of fruiting tends to force the energies of the plant into vegetative lines, and it is not apparent to the writer why this should not be true of the fungi also. On the other hand, Botrytis, which did not fruit, showed nearly or quite as great a stimulation of mycelial development as any other form.

The toxic properties of KOH are probably largely due to the $\bar{O}H$ ion. It is about 94 per cent. ionized at $\frac{n}{12}$ (Ostwald '86), its inhibiting point. Just what proportion of the toxic properties is to be attributed to the remaining 6 per cent. un-ionized KOH we have as yet no means of knowing. Inasmuch, however, as KOH is more highly ionized than HCl at the inhibiting point and is distinctly more toxic, we may safely conclude that the $\bar{O}H$ ion is somewhat more toxic for fungi than ionic $\overset{+}{H}$.

Ammonium hydroxid, NH_4OH; 29 (?), *51*, 83. This hydroxid, in contrast with KOH, is but slightly ionized, $\frac{n}{40}$, the inhibiting concentration, being about 8 per cent. ionized (Kohlrausch '85.) Its high toxic value is then doubtless due very largely to the un-ionized molecule.

Like KOH, although to a less degree, it caused a marked stimulation of mycelial development in many cultures. It is worthy of note that Sterigmatocystis, which is particularly resistant to both ionic $\overset{+}{H}$ and $\bar{O}H$, proves quite susceptible to both acids and hydroxids in the molecular form.

These results with hydroxids are not in harmony with those obtained by Krönig and Paul ('97) with anthrax spores. They found that the bases KOH, NaOH, LiOH and NH_4OH "*disinfect in direct proportion to their degree of ionization*," NH_4OH being practically non-toxic. Anthrax spores have evidently a great specific resistance to this agent, and perhaps even a general comparison would be unfair. Be this as it may, ammonia is without doubt one of the more violent poisons for fungi, far surpassing the mineral acids, copper, cobalt, etc., in toxic properties, and comparing favorably with KCN.

Data as to the effect of hydroxids on the higher plants are rather meager. Kahlenberg and True ('96) found Lupinus to survive in $\frac{n}{200}$ KOH. As ionization is practically complete here, Lupinus is evidently much more resistant to $\bar{O}H$ than to

ionic $\overset{+}{H}$. Bokorny ('88) found that ammonia in $\frac{n}{689}$ concentration, in common with other basic substances, caused the production of granules in the protoplasm of Spirogyra cells, but failed to modify otherwise the normal activities of the cell. Detmoor ('94) found that a 10 per cent. solution of ammonia at first energetically excited the protoplasm of Tradescantia hairs, later producing anæsthesia. Washing with water, however, restored the original characters of the protoplasm. From these data it would seem that hydroxids are more fatal to the molds than to the higher plants.

Formaldehyde, HCHO; 0.553, *1.43*, 2. Formaldehyde, as was anticipated, proved to be one of the most deadly agents tested, being surpassed in this respect by mercury, silver, and the two chromates only. Chemically considered, formaldehyde is a very unstable compound intermediate between methyl alcohol and formic acid, being in fact the intermediate step in the oxidation process by means of which the latter is derived from the former. It is both a reducing and oxidizing agent, and this together with its great instability may account for its extremely toxic properties toward fungi.

To many kinds of protoplasm, including that of the higher animals and perhaps the higher plants, formaldehyde is non-toxic. Instances are on record of persons having drunk a 1 per cent. solution without inconvenience (Arthur '97, p. 21). To the lower animals, however, it is more toxic, $\frac{n}{60}$ being fatal to worms, mollusks and isopods in two hours (Loew '88). Acton ('89), in experiments on the assimilation of organic compounds by green plants, found that while they could use glucose, saccharin, glycerin, etc., they failed to use aldehydes or their derivatives. Cohn ('94) found a 1 per cent. solution very fatal to Spirogyra.

For the fungi, however, there is no doubt that in formaldehyde we have one of our safest, most energetic, and most serviceable poisons. $\frac{n}{512}$ proved fatal to Aspergillus and Penicillium;

$\frac{n}{2048}$, to Sterigmatocystis and Botrytis. Œdocephalum was inhibited by $\frac{n}{16384}$, and killed by $\frac{n}{8192}$. This will perhaps be better appreciated if stated in another way. One part by weight in 273,066 parts beet infusion proved fatal to Œdocephalum, and 1 part in 4,369,066 permitted the germination of but 10 per cent. of the spores in eleven hours (as compared with 95 per cent. in four hours in the checks) and greatly injured the mycelial development. In regard to the other forms, 1 part to 273,066, although greatly retarding germination, caused a distinct stimulation of mycelial growth on the second day. These stimulated cultures resembled those growing in media containing alcohol.

Very interesting in this connection are the theories regarding the synthesis of starch in green plants, and of the proteids in the fungi (Kozlowski '99), in both of which formaldehyde has long been regarded as forming a very important step. These theories, particularly that in regard to the synthesis of proteids in the fungi, challenge further careful investigation. It seems, *prima facie*, inconceivable that a compound markedly injurious to a plant when present in the almost infinite dilution of one part of weight in 4,369,000 parts nutrient medium, as is the case of Œdocephalum, should be formed by the protoplasm of that plant and be used again in the synthesis of its proteids, as must be the case if our theories be correct.

In regard to the nature of its toxic action we have few data. It is believed to act upon the propeptones and the albumins, affording compounds which are not readily soluble (Davenport '97).

Ethyl alcohol, C_2H_5OH; 717, *3686*, 8602. Alcohol when contrasted with formaldehyde, is apparently non-toxic. One molecule of formaldehyde has powers of inhibiting germination of fungus spores equal to those of 2600 molecules of alcohol. The contrast in their killing powers is even greater, being as 1 : 4300! Yet, alcohol is a distinct poison to the protoplasm of plants (Tsukamoto '95), being used, in fact, quite extensively as a fixing

agent for tissues, and even more widely as a preservative. It inhibits germination in the mold spores used at an average concentration of $\frac{n}{1.3}$, and is fatal to all except Penicillium in $\frac{4n}{1}$. Five per cent. of the spores of Penicillium survived immersion in this concentration (17.6 per cent.) for 72 hours at 28° C. All, however, were killed by $\frac{8n}{1}$ which was the greatest concentration required to kill in the case of any agent tested.

The presence of alcohol in $\frac{n}{8}$ and $\frac{n}{16}$ concentration distinctly retarded germination of Sterigmatocystis and Botrytis. No retardation was noticed with Aspergillus or Œdocephalum. The latter, indeed, showed some evidence of acceleration of germination in $\frac{n}{16}$ and $\frac{n}{32}$ concentrations, but it was not sufficiently marked to be certainly stated. The stimulation of mycelial development and retardation of fruiting in $\frac{n}{4}$ was very marked with Aspergillus and Sterigmatocystis. Œdocephalum and Penicillium showed some stimulation of mycelial development and a slight retardation of fruiting in this concentration. Botrytis produced its heaviest mycelium in $\frac{n}{16}$ and $\frac{n}{32}$.

The change that a plant may undergo as it grows older, in the character of its election of foods is of great interest to physiologists (Davenport '99, p. 333). Duclaux ('89) found that while alcohol restrains or arrests germination in mold spores, it is made use of almost as abundantly as sugar by the adult plant. My results would seem to support this view. It may be possible, however, that alcohol acts as a stimulant rather than as a food, as is the case with zinc sulfate, and other non-nourishing compounds. Richards ('97) found that the addition of .0035 per cent. $Zn\,SO_4$ to a culture medium in which *Aspergillus niger* was growing doubled the dry weight of the mycelium. .008 per cent. $Zn\,SO_4$ similarly added to a flask culture of Botrytis caused a production of quadruple the normal weight of mycelium. We cannot suppose that the small amount of zinc present

in itself caused the greater growth by supplying nourishment, Zn not being a necessary or desirable element in a nutrient medium for fungi. Richards interpreted the function of the zinc to be that of a stimulant rather than a food. May it not be that alcohol performs a similar stimulating function, rather than that it produces an acceleration of growth by nourishment?

Whatever may be the correct explanation of the influence of alcohol on the development of the mold fungi, it seems to be demonstrated that the protoplasm of the molds is more sensitive in the conidial stage to the influence of this and most other deleterious agents than at any other stage in their development.

Potassium cyanid, KCN; 2.2, *25.6*, 77. Potassium cyanid in aqueous solution is very unstable. A solution of 24 per cent. KCN in pure water was prepared by the chemist, and on being used within three hours of titration gave the following critical points with Aspergillus and Penicillium:

Aspergillus in $\frac{n}{128}$ grew, in $\frac{n}{64}$ failed.

Penicillium in $\frac{n}{128}$ grew, in $\frac{n}{64}$ failed.

Ten days later this stock solution was again tested, having been kept in a dark cupboard at ordinary laboratory temperature in the meantime, with the following result:

Aspergillus in $\frac{n}{32}$ grew, in $\frac{n}{16}$ failed.

Penicillium in $\frac{n}{64}$ grew, in $\frac{n}{32}$ failed.

A solution in beet infusion containing $\frac{n}{8}$ $C\bar{N}$ was then made up from the stock solution and placed in the dark at a constant temperature of 28° C. for 10 days longer and again tested. The critical points were then as follows:

Aspergillus in $\frac{n}{16}$ grew, in $\frac{n}{8}$ failed.

Penicillium in $\frac{n}{16}$ grew, in $\frac{n}{8}$ failed.

From these data we learn that a 24 per cent. aqueous solution of KCN deteriorates so that at the end of ten days it has but little more than one fourth its former toxic value. Made up in beet infusion at $\frac{n}{8}$ concentration and kept ten days longer it retains but one eighth of its original toxic value. All cultures with KCN, reported in this paper, except those noted above, were made up within four hours of titration of the stock solution.

It will be seen by reference to the charts that KCN in solution has almost exactly nine times the toxic effect of ionic $\overset{+}{H}$. KCN in the concentrations used is quite highly ionized (Kohlrausch '79), but in trying to approximate the toxic value of the $C\overline{N}$ ion, the fact that a certain amount of hydrolysis takes place in aqueous solutions of this salt, with a corresponding formation of the deadly HCN, must not be overlooked. According to the data worked out by Shields ('93) and what we already know of the properties of HCN, approximately 15 per cent. of the total toxic value of these solutions must be attributed to the HCN present. This would give something under $8\overset{+}{H}$ as the value of the $C\overline{N}$ ion.

KCN retarded germination and early development in all forms. In this, as with many other agents, those cultures not greatly injured soon overcame the effect of the poison and grew and fruited normally. No marked retardation of fruiting nor unusual development of mycelium was noted. Sterigmatocystis, in so many cases highly resistant, proved equally sensitive with Œdocephalum, both being inhibited by $\frac{n}{128}$ and killed by $\frac{n}{64}$.

Kahlenberg and True ('96) found that towards Lupinus it has the value of $1\overset{+}{H}$ only. They also show that in the cases of potassium ferro- and ferri-cyanid the iron and $C\overline{N}$ radical form complex ions, the toxic value of which is far less than that of the $C\overline{N}$ ions.

Mercuric chlorid.—$HgCl_2$; 0.0258, *0.281*, 0.331. This proved the most fatal compound tested, leading silver nitrate by a narrow margin.

It is of interest to note that although only very slightly volatile at ordinary temperatures, and doubtless less so in aqueous solution, mercuric chlorid is sufficiently volatile even in dilute aqueous solution at 28°C. to be distinctly toxic. This was demonstrated by placing a few drops of a very dilute solution in the bottoms of cells containing hanging drops of pure beet infusion inoculated with mold spores. The germination of the spores was inhibited. Mycologists have frequently reported failure to germinate spores in cells which had been sterilized by rinsing in a dilute solution of $HgCl_2$. These failures were doubtless due to the volatile properties of this agent together with its extremely deadly character.

Botrytis proved particularly sensitive to this agent, $\frac{n}{65536}$ proving fatal. Penicillium failed to show its usual high relative resistance, being killed by $\frac{n}{4096}$, the same concentration as proved fatal to Aspergillus and Sterigmatocystis. Toward bacteria it is also extremely fatal, $\frac{n}{70000}$ in nutritive bouillon preventing the development of the splenic fever bacterium (Davenport '97, p. 14). The data regarding its influence on the higher plants are meager. Kahlenberg and True ('96) found $\frac{n}{6400}$ fatal to Lupinus, while it survived in $\frac{n}{12800}$. This is unexpectedly low, being in fact but double the toxic value of HCl. For the molds its average value will be seen to be over 800 times that of HCl.

Silver nitrate, $AgNO_3$; 0.0125, *0.375*, 0.375. Almost, if not altogether, as violent a poison as mercury, silver stands with it at the head of the list of toxic agents tested. Among the poisons for molds tested, it is comparable with mercury alone among the metals, and with the chromate and dichromate anions and formaldehyde only among the other agents. As is the case with bacteria (Davenport '97, p. 14) toward the molds silver is frequently a more violent poison than mercury. Of the five molds used Œdocephalum and Penicillium proved more susceptible to

silver, while Aspergillus and Botrytis were more susceptible to mercury. The fifth form, Sterigmatocystis, had an equal resistance to both. The extraordinarily small quantity of mercury required to kill Botrytis, however, left the honors with mercury as the more deadly agent.

A very striking contrast in specific resistances is afforded by Botrytis and Penicillium in solutions of these agents. One sixteenth the amount of $HgCl_2$ necessary to kill Penicillium was fatal to Botrytis. With $AgNO_3$ one fourth the concentration required to kill Botrytis was fatal to Penicillium. Or, putting it another way, $AgNO_3$ has eight times the toxic value of $HgCl_2$ toward Penicillium, while the exact converse is true with Botrytis, $HgCl_2$ being eight times as effective as $AgNO_3$ to this form. The low resistance of Penicillium to silver is quite striking, $\frac{n}{32768}$ proving fatal. In one other case only (H_2O_2) did Penicillium show a lower resistance than Botrytis.

Toward splenic fever bacteria gold is the only other metal comparable in toxic properties with mercury and silver (Davenport '97, p. 14). Toward phanerogams silver is much more toxic than mercury, $\frac{n}{102400}$ being fatal to Zea, and $\frac{n}{204800}$ to Lupinus (Heald '96, p. 152). At the concentrations used the $AgNO_3$ would be practically entirely ionized (Kohlrausch, '85).

Cadmium nitrate, $Cd(NO_3)_2$; 0.075, *6.1*, 24. Ranking closely with silver as a poison for the higher plants, cadmium proves to be very highly toxic to the mold fungi. Perhaps the most marked feature in the cultures with cadmium was the very wide range between the killing point and the point where development was only noticeably injured. It will be noticed that the ratio of its coefficient of injury to that of $HgCl_2$ is less than 3 : 1, while the ratio between their death points is 75 : 1. A second striking feature of the toxicity of cadmium is the great variation in the specific resistances of the different forms. $\frac{n}{4096}$ proved fatal to Botrytis, but $\frac{n}{32}$ was required to kill Sterig-

matocystis. Penicillium spores failed again to show their usually high resistance, $\frac{n}{256}$ proving fatal, a concentration in which the usually sensitive Œdocephalum actually germinated 95 per cent. and matured a few fruits.

Molisch ('94) was one of the first to record the toxic properties of cadmium to plants. He found $\frac{n}{33}$ fatal to Aspergillus when experimenting with various metals in an endeavor to find a substitute for calcium in nutrient media. $\frac{n}{64}$ proved fatal to the form of Aspergillus used by the writer.

At its average inhibiting concentration cadmium nitrate would be about 90 per cent. ionized (Grotrian, '83).

OXIDIZING AGENTS.

Potassium dichromate, $K_2Cr_2O_7$; 0.094, *0.3*, 1.25.
Potassium chromate, K_2CrO_4; 0.156, *0.4*, 2.25.

These salts at the dilutions at which they are effective are doubtless practically entirely ionized (Ostwald, '88).

As poisons for the molds they rank, as already mentioned, with formaldehyde, silver, and mercury. The anion of the dichromate, $\bar{Cr}_2O_7^{=}$ has a toxic value of about 770 $\dot{H}$; that of the chromate 575 $\dot{H}$. This may indicate some relation between their oxidizing powers and their toxicity.

The effects of these salts in concentrations permitting development of the fungi were very similar, and resembled that of H_2O_2. Retardation of germination in the cultures approaching the inhibiting point was noticed in all forms with both agents, but it was not nearly so well marked as it is in most cases. Another feature was the fact that every culture that germinated any spores developed some conidia within forty-eight hours.

Toward the higher plants these anions seem to be relatively much less toxic. Lupinus survives in a $\frac{n}{6400}$ solution of H_2CrO_4, the same concentration as permitted growth with HCl (Kahlenberg and True, '96). Toward the algæ (Loew, '93), however,

both these anions are strongly toxic, $\frac{n}{295}$ $K_2Cr_2O_7$ being fatal to Spirogyra in a few hours.

Hydrogen peroxid, H_2O_2; 38, *105*, 127. This agent had an effect on the molds very similar to that described for the chromates. With it, however, the characteristics given for the chromates were somewhat intensified. Germination was but slightly retarded in most forms in $\frac{n}{312}$ concentration, and Œdocephalum actually showed a higher percentage germinated in these cultures at four hours than in the checks. The difference, however, was not sufficient to establish the conclusion that the H_2O_2 accelerated germination. That this concentration accelerated early mycelial growth with this form was undoubtedly established. At four hours the average length of the germ tubes in $\frac{n}{312}$ concentration was 120μ as compared with 40μ in the checks. At seven hours they were 310μ and 115μ respectively. $\frac{n}{156}$, the limiting culture for this form, made the best mycelial development and matured the heaviest crop of conidia in the set. The characteristic already mentioned for the chromates regarding fruiting in the cultures was even more marked with this agent. Every culture that produced even the scantiest mycelium presently developed at least two or three all but normal conidiophores.

In regard to the action of this agent on other organisms, the data are meager and conflicting. Miquel ('83) places it third in disinfecting properties of all agents used by him. In his results it is rated above both $HgCl_2$ and $AgNO_3$, being given as antiseptic in dilution of 1 part to 20,000. This is certainly too high an estimate. Sternberg ('92) found it to have a comparatively low toxic value for bacteria. One in 1000 kills ordinary water bacteria, cholera, and typhoid (Altehofer '90). This would be about $\frac{n}{33}$, or a 3 to 4 per cent. solution of the ordinary 10-volume commercial article. It will be seen that this agrees fairly closely with its toxic properties for the molds, $\frac{n}{39}$

being fatal to Sterigmatocystis and $\frac{n}{78}$ to Œdocephalum. The others are more resistant. It is, however, more toxic to algæ (Bokorny, '86) than to molds, and Ciliata are even more susceptible. Paneth ('89) found a .005 per cent. solution to be the limiting line for the latter.

Commercial preparations of H_2O_2 vary very greatly in the amount of H_2O_2 in solution. A true ten-volume solution should yield, when fully decomposed, ten volumes of O, and should contain by weight 3.04 per cent. H_2O_2. The preparation used in this study although "fully guaranteed," etc., contained but 2.59 per cent. H_2O_2 on being tested.

SULFATES OF THE STRONGLY-TOXIC METALS.

These salts are arranged in the order of their toxic properties towards molds in the following list:

Nickelous sulfate, $NiSO_4$; 4.8, *33.6*, 1155.
Cobaltous sulfate, $CoSO_4$; 6, *57.6*, 389.
Ferrous sulfate, $FeSO_4$; 14.4, *115*, 2150.
Copper sulfate, $CuSO_4$; 8.4, *131.2*, 582.
(Copper nitrate, $Cu(NO_3)_2$; 8.4, *134*, 634.)
Zinc sulfate, $ZnSO_4$; 26.4, *602*, 3072.

The data regarding the ionizations of these salts are rather meager. They are, however, not greatly different in ionization at similar concentrations. This is about 40 per cent. to 44 per cent. at $\frac{n}{20}$ concentration (Whetham, '95, pp. 218–276).

Nickelous sulfate.—The different molds exhibited more variations in their specific resistance to this agent in regard to the death point than was observed with any other. $\frac{n}{128}$ proved fatal to Botrytis, while Aspergillus failed to lose its vitality in a normal solution (containing over 13 per cent. anhydrous $NiSO_4$) for 48 hours. Much less variation was shown in its inhibiting powers. Aspergillus and Penicillium germinated in $\frac{n}{64}$; $\frac{n}{256}$ inhibited Botrytis. The fact that 32 times the strength

which inhibited the spores of Aspergillus failed to kill them was nowhere else paralleled with this form and but once surpassed by Penicillium.

Cobaltous sulfate stands in second place in this group as an inhibiting agent, but, as will be seen, it is relatively much more powerful as a disinfectant. Penicillium, however, showed its usual high powers of resistance in this respect. Inhibited by $\frac{n}{64}$, $\frac{n}{2}$ was required to kill.

Ferrous sulfate.—Iron, a necessary element for the nutrition of the molds (Molisch, '94) in common with all other plants, in excess proves to be a very strongly toxic agent, surpassing in this respect that king of modern fungicides, copper. With the exception of nickel as noted above, iron showed a greater difference between the average concentration required to inhibit the spores and the concentration required to kill than any other agent. Botrytis, as usual, showed less variation in this respect than the other forms, but even with it one eighth the fatal concentration inhibited germination. Œdocephalum showed the greatest resistance to this agent both as regards inhibition of germination and killing of the spores. This was the only agent with which it had a higher specific resistance than any other form.

Copper sulfate and nitrate.—These salts proved to be quite similar in toxic properties, as may be noticed by a glance at the diagrams, p. 312. The nitrate, however, is much more highly ionized at the critical concentrations; hence we judge that the un-ionized molecule $CuSO_4$ has a toxic value not greatly different from ionic $^{+}Cu^{+}$.

Penicillium, although inhibited by $\frac{n}{64}$ $Cu(NO_3)_2$ and $\frac{n}{128}$ $CuSO_4$, required a $\frac{n}{1}$ concentration in both cases to kill the spores. This certainly shows great resistance to these agents as compared with the other molds. It, however, appears insignificant when contrasted with many of the results gotten by other workers. $\frac{n}{128}$

$CuSO_4$, which effectually inhibited germination in the form used by the writer, contains about 0.1 per cent. $CuSO_4$. De Seynes ('95) reports growing cultures of *Penicillium glaucum*, gotten from different sources, in solutions containing 2 to 9.5 per cent. $CuSO_4$. Cultures grown on the stronger concentrations bore red spores. Pfeffer ('81) reports finding Penicillium growing on a concentrated solution of $CuSO_4$. Manasein (*fide* Loew '93) finds from his experiments that this salt must be present in a .25 per cent. concentration before it has any appreciable effect on this fungus. Others might be quoted, but sufficient has been said to indicate the possibilities yet to be investigated of the acclimatization of fungi (and other plants) to chemical agents (Davenport and Neal, '96).

Zinc sulfate.—Inasmuch as zinc chloride is used very extensively for impregnating railroad ties to prevent attacks of wood-destroying fungi (Roth, '95), it was a surprise to find it having so low a toxic value, particularly when it is recalled that one of the molds tested, Penicillium, is one of the enemies of the wooden ties (Ward, '98). Koch ('81) finds the chlorid and the sulfate to have practically the same disinfecting power. Towards Aspergillus we may say that zinc is non-toxic, the spores surviving an immersion of 48 hours in a $\frac{2n}{1}$ (27 per cent. anhydrous $ZnSO_4$) concentration. In a $\frac{n}{2}$ concentration (7 per cent.) 25 per cent. of the spores germinated and grew slowly. The mycelium produced was very irregular and closely septate.

Strychnin sulfate, $C_{21}H_{22}N_2O_2H_2SO_4$. This alkaloid, the only one experimented upon, was dissolved in the slightly acid beet infusion until a saturated solution was obtained. This was found to have a content of 4.31 per cent. strychnin sulfate. This in terms of a normal solution would be about $\frac{n}{7}$, a normal solution of this substance requiring over 30 per cent. on account of its very large and heavy molecule.

Sterigmatocystis grew and fruited normally in this saturated solution, although germination and early growth were retarded.

Aspergillus and Œdocephalum also grew and fruited, but the cultures were much behind the checks. Penicillium was inhibited by $\frac{n}{28}$, but was not killed by the saturated solution. Botrytis was also inhibited by $\frac{n}{28}$, and was killed by $\frac{n}{14}$. That the solution used was completely saturated was shown by the appearance of numerous microscopic crystals in some of the hanging-drops which were exposed for a few moments to the air, the evaporation from the culture medium causing some of the strychnin to crystallize out. The molds, however, continued to thrive in these cultures, their hyphæ growing among the crystals.

Of its effect on plants in general we have few data. Davenport ('97) mentions that it kills the protoplasm of Drosera tentacles, and hinders the development of peas, corn, and lupines. The injurious concentrations, however, are not mentioned. Much interesting work has been done on Protozoa (Schürmayer '90) by various workers. The results of these studies as well as those presented here for the molds are in harmony with the theory of Loew ('93) that the action of alkaloids is chiefly confined to the plasma of the ganglion cells. Fungi and bacteria having no differentiation of nerve protoplasm are practically unharmed by this agent.

Potassium iodid, bromid, and chlorid.—These salts proved to have a very low toxic value. A complete series of cultures with the five molds was made up with the iodid only. Its coefficients were determined to be 384, *2457*, and 4915. $\frac{n}{1}$ inhibited all except Aspergillus, $\frac{2n}{1}$ was fatal to all except Penicillium. As the potassium salts of the haloid acids are all quite highly ionized, an attempt was made to determine the relative toxic properties of the *ionic* halogen elements. Aspergillus and Œdocephalum were used. To these molds ionic $\bar{I}$ proved doubly toxic as compared with $\bar{Cl}$. $\bar{Br}$ occupied an intermediate position, being very slightly more toxic than ionic $\bar{Cl}$.

Sodium salicylate $C_6H_4{<}^{OH}_{COONa}$; 24, *182*, 182. It was

thought desirable to test this salt on account of its wide use as a preservative in laboratories and elsewhere. It proved somewhat more toxic than HCl, but not so fatal as HNO_3. Œdocephalum was quite susceptible to its influence, $\frac{n}{128}$ being fatal. It is not, however, of much value as a disinfectant, over 1 per cent. being necessary to prevent the development of molds.

In the following table the various agents are arranged in the order of their toxic properties as shown by their powers of inhibiting germination of the spores of the five species of molds used. The fifth column gives in round numbers the number of molecules of each substance required to produce an inhibiting effect equal to that *of one molecule of mercuric chlorid.* The coefficients have the usual value of x in the expression, $\frac{x}{2048}$ of $\frac{n}{1}$.

TABLE III.

Agent	Formula	Coefficient of injury	Coefficient of inhibition	Coefficient of death-point	Ratio
Mercuric chlorid	$HgCl_2$	.026	.281	.331	1.
Potassium dichromate	$K_2Cr_2O_7$	.094	.3	1 25	1.1
Silver nitrate	$AgNO_3$	.013	.375	.275	1.3
Potassium chromate	K_2CrO_4	.156	.4	2.25	1.4
Formaldehyde	HCHO	.553	1.43	2.	5.
Hydrocyanic acid	HCN	.365	3.	20.	11.
Cadmium nitrate	$Cd(NO_3)_2$	.075	6.1	24.	22.
Potassium cyanid	KCN	2.2	25.6	77.	91.
Nickelous sulfate	$NiSO_4$	4.8	33.6	1155.	120.
Ammonium hydroxid	NH_4OH	8.	51.	83.	182.
Cobaltous sulfate	$CoSO_4$	6.	57.6	389.	206.
Monochloracetic acid	CH_2ClCO_2H	8.8	58.	64.	207.
Dichloracetic acid	$CHCl_2CO_2H$	10.4	64.	64.	229.
Acetic acid	CH_3CO_2H	25.6	83.	314.	296.
Trichloracetic acid	CCl_3CO_2H	37.	90.	115.	321.
Hydrogen peroxid	H_2O_2	38.	105.	127.	375.
Ferrous sulfate	$FeSO_4$	14.4	115.	2150.	411.
Copper sulfate	$CuSO_4$	8.4	131.	582.	468.
Copper nitrate	$Cu(NO_3)_2$	8.4	134.	634.	479.
Nitric acid	HNO_3	48.	141.	384.	503.
Potassium hydroxid	KOH	19. / 77.	166.	282.	593.
Sodium salicylate	$C_6H_4{<}^{OH}_{CO_2Na}$	24.	182.	182.	650.
Sulfuric acid	$\frac{1}{2}H_2SO_4$	61.	205.	589.	732.
Hydrochloric acid	HCl	70.	230.	614.	821.
Zinc sulfate	$ZnSO_4$	26.4	602.	3072.	2150.
Strychnin sulfate	$C_{12}H_{22}N_2O_2H_2SO_4$	179.			8775.
Potassium iodid	KI	384.	2457.	4915.	13164.
Ethyl alcohol	C_2H_5OH	717.	3686.	8602.	

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4
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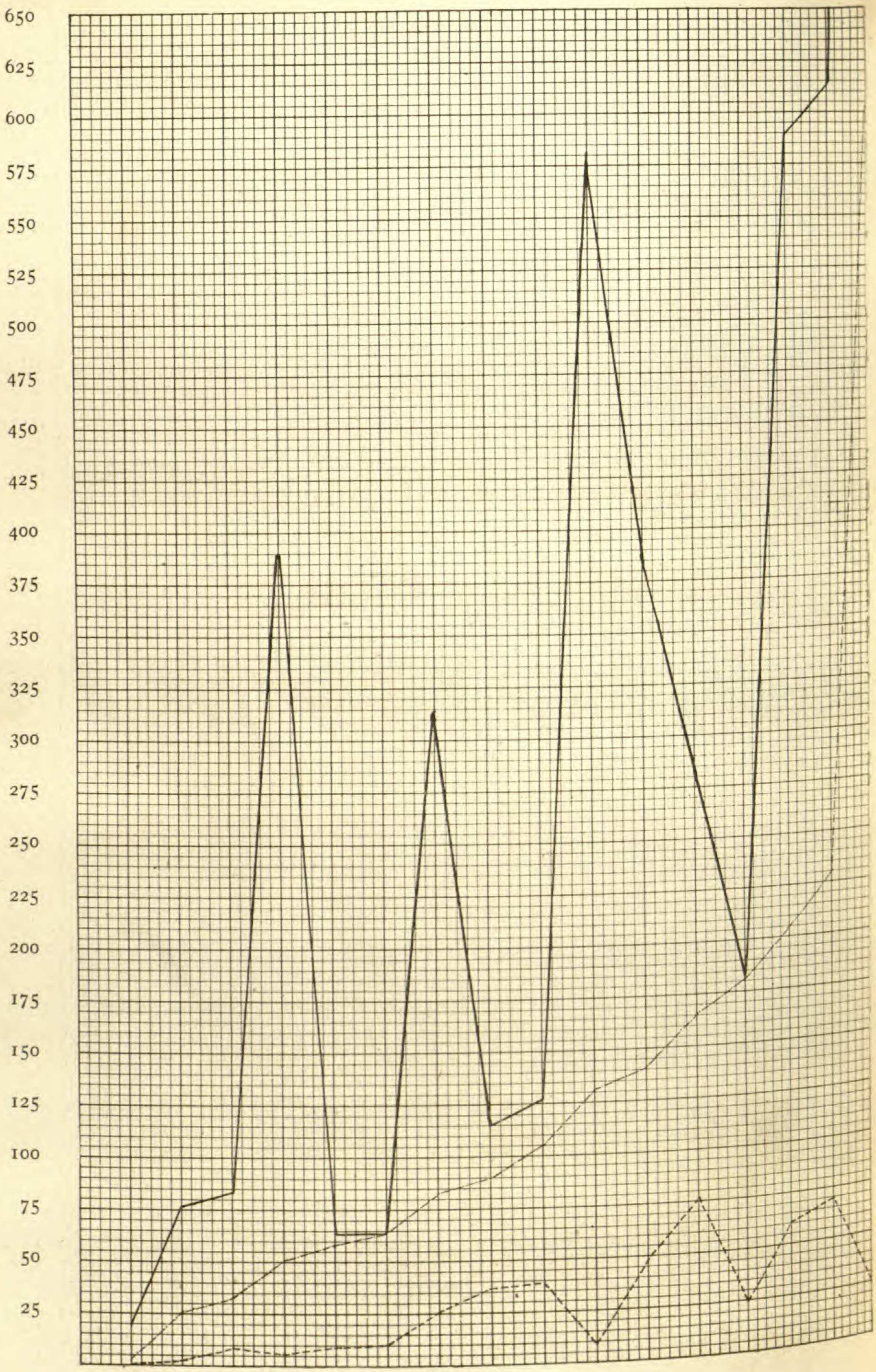
2
1
HgCl2
K2Cr2O7
AgNO3
K2CrO4
HCHO
HCN
Cd(NO3)2
KCN
NiSO4
NH4OH4
CoSO4
CH2ClCO2H
CHCl2CO2H
CCl3CO2H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HCN
KCN
NH_4OH
$CoSO_4$
CH_2ClCO_2H
$CHCl_2CO_2H$
CH_3CO_2H
CCl_3CO_2H
H_2O_2
$CuSO_4$
HNO_3
KOH
$C_6H_4<^{OH}_{COONa}$
$\frac{1}{2}H_2SO_4$
HCl
$ZnSO_4$

Diagrams IX and X are simply a graphic representation of the more important features of this table. The values of the abscissæ in each case $= x$ in the expression $\frac{x}{2048}$ of a normal solution.

SUMMARY.

1. Fungi are in general much more resistant to most deleterious agents than the higher plants. In the case of the mineral acids a concentration of from two to four hundred times the strength fatal to the higher plants is required to inhibit the germination of mold spores under favorable conditions.

2. Different species of fungi present great differences of resistance to many agents. Of the agents tested in this study, $NiSO_4$ permitted the greatest specific variation and dichloracetic acid the least.

3. Particular forms of the same species present very different powers of resistance, depending probably on previous environment.

4. Individual spores taken from the same pure culture often present considerable variation in resistance.

5. The five forms used were found to be increasingly resistant to the toxic action of acids in the following order: Œdocephalum, Botrytis, Penicillium, Aspergillus, and Sterigmatocystis.

6. Toward all the agents tested they proved increasingly resistant in the following order: Botrytis, Œdocephalum, Penicillium, Sterigmatocystis, Aspergillus.

7. Œdocephalum and Botrytis, although on the average the most susceptible to the various agents, have great specific resistances to certain agents. See $FeSO_4$, KI, and alcohol.

8. Tests made with media not well suited for the normal development of the fungi tested will give a correct value for the *killing concentration*, but the data regarding the point of inhibition of germination are not of value.

9. Tests of the toxic value of solutions are unreliable when made in hanging-drop cultures where water was used in the

bottom of the cell instead of a solution similar in composition to that forming the hanging-drop. The variation in the toxic value indicated from the actual value will depend on the *vapor pressures* of the solutions used. Volatile—especially highly volatile—and hygroscopic solutions will show the greatest error.

10. Many deleterious agents which at certain concentrations retard germination and early growth, afterwards cause a great acceleration of mycelial development in these retarded cultures. This abnormal development of mycelium is usually accompanied by retardation of fruiting.

11. In the conidial stage the protoplasm of molds is in general more sensitive to the action of deleterious agents than at any other stage in their life history.

12. The effect of the different deleterious agents on the appearance of the mycelium is very varied and often quite characteristic.

13. One is not justified in drawing any conclusions as to the killing powers of an agent from its effect in inhibiting the germination of the spores.

14. The hydroxyl group $\bar{O}H$ is rather more toxic to molds than ionic $\overset{+}{H}$.

15. The toxic value of the halogens, Cl, Br, and I, in the *ionic* state, increases somewhat in the order of increasing atomic weight.

16. The cyanogen radical is a very powerful poison to fungi, KCN having nine times the toxic value of HCl.

17. Mercuric chlorid and silver nitrate are about equally toxic to molds; and are followed in toxic properties by potassium dichromate and chromate, and formaldehyde.

18. Strychnin and hydrocyanic acid, both extremely fatal poisons to the higher animals, and both supposed to act on the protoplasm of the nerve cells, react very differently toward fungi. To the molds strychnin is practically non-toxic, whereas hydrocyanic acid is a very violent poison.

19. Nickel, cobalt, iron, copper, and zinc inhibit mold spores in the order named. Zinc is much less toxic than the others.

20. That an element is necessary for the nutrition of a plant does not indicate whether it would or would not be a poison in greater concentration. See iron, oxygen, etc.

21. That an element is not necessary for the normal development of a plant does not imply that it would be injurious even in relatively great concentration. See chlorin, calcium, etc.

22. The ionization of the molecule of electrolytes in aqueous solution has a very important bearing on the study of the physiology of poisons. It is of especial value in determining the element or group of elements in a compound to which its toxic properties are to be attributed.

23. In this study no new evidence has been adduced supporting the theory that the chemical activities of a substance are due wholly or chiefly to the ionized portion.

24. Evidence has been adduced to the effect that in the case of several acids ionization *lessens* the chemical activities toward the substances involved in the life processes of the plant.

25. In the case of the eight acids investigated six were found to be much more toxic in the molecular form than after ionization. The toxic properties of the un-ionized molecules vary from approximately 2.8 times that of ionic $\overset{+}{H}$ in the case of acetic acid to 76.6 times that of $\overset{+}{H}$ in hydrocyanic acid.

26. The substitution of Cl for H in the acetic acid radical has a double effect. In the first place it increases the toxicity of the un-ionized molecules to a greater or less extent depending on the number of H atoms so replaced. In the second place, it increases the ionization of the acid. The amount of the ionization is also dependent on the amount of H so replaced, being greatest, as are the toxic properties of the whole molecules, when all three H atoms have been replaced by Cl.

27. These two factors to a great extent counterbalance each other. Which has the greater influence in any given solution depends altogether on the concentration, the increased toxicity of the molecules having the predominating influence at the greater concentrations, and the ionization being more effective at the greater dilutions.

28. At the concentrations inhibiting fungus spores mono- and dichloracetic acids are more influenced by the increased toxic properties of the molecule, and trichloracetic by the ionization. The former in $\frac{n}{35}$ and $\frac{n}{32}$ concentration are respectively increased in toxicity 40 per cent. and 30 per cent. over the original acetic. Trichloracetic, on the contrary, at $\frac{n}{22.8}$ suffers a reduction of 10 per cent. in toxic properties as compared with the original acetic.

29. The anions of the mineral acids, HCl, HNO_3, and H_2SO_4, have a low toxic value for fungi, having less than one thirty-second that of ionic H.

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